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Phosphorus Fractions in Developing Seeds of Four Low Phytate Barley (*Hordeum vulgare* L.) Genotypes

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ABSTRACT

***Low phytic acid* (*lpa*) crops have reductions in the amount of seed phytic acid (*myo*-inositol hexa*kis*phosphate, $InsP_6$) and increases in inorganic phosphorus (Pi) with little change to the amount of total seed P. In this study, four barley *lpa* genotypes (*lpa*1–2, *lpa*2–1, *lpa*3–1, and M955), backcross wild-type (*wt*) sib-selections, and original parental line 'Harrington' were grown in the field over 2 yr. Developing seed was harvested once a week for 3 wk and then again at physiological maturity, and the seeds assayed for levels of total P, Pi, and phytic acid P. Total phosphorus concentration showed no consistent differences between the *lpa* genotypes and Harrington. Inorganic P declined during development in the *wt* genotypes; however in *lpa* genotypes, inorganic P declined during the first few weeks of development, and then increased from 24 d to maturity. Phytic acid concentration increased steadily during development for the *wt* lines and barley *lpa*1–1 and *lpa*2–1, although the increase was much slower in the *lpa* lines. The *lpa*3–1 and M955 had very little $InsP_6$ accumulation until later in development, with little to no increase in the amount of phytic acid in mature seed of M955 compared to the developing seeds of *lpa*3–1. This information is useful in understanding timing of phosphorus accumulation in seeds, as well as the nature of the *low phytic acid* mutation in seed development.**

PHYTIC ACID (*myo*-inositol hexa*kis*phosphate, $InsP_6$) is the most abundant phosphorus-containing molecule in seeds. Phytic acid within the seed is often complexed with other minerals to form salts labeled phytate, which are primarily sequestered in the aluerone and embryo tissues of cereal seeds. It typically comprises from 60% to 80% of total seed phosphorus in mature seeds (Lott et al., 2000). Phytate is thought to play an important part in phosphorus and mineral homeostasis during seed development (Otegui et al., 2002). Additionally, phytic acid and inositol derivatives are important to various important plant functions. For instance, phytic acid is known to act in diverse functions such as regulating stomatal closure (Lemtiri-Chlieh et al., 2000, 2003) and ameliorating effects of soil cation toxicity (Van Steveninck et al., 1987). In other eukaryotic systems, phytic acid is important in G protein mediated signaling (Sasakawa et al., 1995) and increasing evidence indicates it contributes to chemoprevention of cancer (Vucenik and Shamsuddin, 2003). Inositol derivatives are important in other processes including cell wall biosynthesis, stress response, and oligosaccharide synthesis (Reviewed in Loewus and Murthy, 2000).

Recent investigations have identified phytic acid as a significant factor in phosphorous cycling and agricultural systems (Brinch-Pedersen et al., 2002). Natural variation in phytic acid and phosphorus levels in seeds has been demonstrated in *Arabidopsis thaliana* (Bentsink et al., 2003). Reductions in phytic acid are desirable from a nutritional standpoint, as phytic acid is a potent chelator of essential minerals, particularly, K^+, Ca^{2+}, Fe^{2+} and Zn^{2+}. In addition, phytate is indigestible and largely excreted by non-ruminant animals, and is an important component of phosphorus pollution in the environment (Raboy et al., 2001). To this end, efforts have been made to reduce the level of phytic acid in seeds using transgenics and mutagenesis. Consequently various *low phytic acid* (*lpa*) mutants have been identified in a number of species. *Low phytic acid* mutants are characterized by reduced phytic acid P, and increased inorganic P. Low phytate genotypes have been identified in maize (*Zea mays* L.; Raboy et al., 2000), barley (*Hordeum vulgare* L.; Larson et al., 1998; Rasmussen and Hatzack, 1998; Dorsch et al., 2003), rice (*Oryza sativa* L.; Larson et al., 2000), soybean (*Glycine max* L. Merr.; Wilcox et al., 2000; Hitz et al., 2002), and wheat (*Triticum aestivum* L.; Guttieri et al., 2004). Null phytic acid mutants have not been reported, suggesting that this metabolite is essential in plant development.

As phytic acid represents a significant portion of total seed P, the accumulation of phytate and inositol phosphates has been studied in different plant species. Raboy and Dickinson (1987) recorded the accumulation of phytic acid, inorganic P, and total P in wild-type (*wt*) soybean lines. Raboy et al. (2000) did similar work with the maize *lpa*1–1, *lpa*2–1, and *wt* lines. In both studies, phytate accumulates gradually during seed development. Inorganic P concentration decreases during seed development, and total P levels remain relatively consistent. The maize *lpa* genotypes had little to no increase in phytate, and inorganic P concentration was high and did not decrease during development.

While the final levels and partitions of P forms in barley are well documented, it is unclear how seed phosphorus accumulates in developing seeds of different barley low phytic acid genotypes. Similarly, it is unclear when in seed development the expression of the *lpa* phenotype is initiated in barley. The objectives of this study are to investigate the accumulation of phosphorus in developing seeds of four barley *lpa* genotypes with a range of reductions in phytic acid.

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Abbreviations: DAA, days after anthesis; $InsP_6$, *myo*-inositol hexa*kis*phosphate; *lpa*, low phytic acid; P, phosphorus; Pi, inorganic phosphorus; PA, phytic acid; *wt*, wild-type.

MATERIALS AND METHODS

Plant Material

Four barley *low phytic acid* genotypes (*lpa*1–1, *lpa*2–1, *lpa*3–1 and M955) and the *wt* recurrent cultivar 'Harrington' (Harvey and Rossnagel, 1984) were selected to represent a range of reductions in phytic acid as well as types of reduction (Table 1). These mutants were described by Larson et al. (1998), Raboy et al. (2001), Dorsch et al. (2003), and Ockenden et al. (2004).

Each of the four original *lpa* genotypes were backcrossed to the original parent cultivar Harrington to create BC_2 families for M955, BC_3 families for *lpa*2–1 and *lpa3–1*, and BC_4 families for *lpa*1–1. From these families, three phenotypically *lpa* and three phenotypically *wt* sib-lines were selected and used for this study. Each of the sib-selections and the cultivar Harrington were planted in a randomized complete block design with two replications over 2 yr (2003 and 2004), totaling 25 entries per replication. Plots consisted of seven row plots of the dimensions 1m by 2m. Cultivation and fertility practices were the same as commonly used by barley producers in Idaho's irrigated production area (Robertson and Stark, 2003). Developing spikes were harvested beginning 10 d after anthesis (DAA) and continuing at 17 and 24 DAA. Approximately 20 spikes were harvested each day, and were selected to represent the mean developmental stage of the plot. The developmental stages of the seeds were as follows; Harvest 1– watery ripe stage 10 DAA (Zadoks stage 71), Harvest 2– milk stage 17 DAA (Zadoks stage 77), Harvest 3– soft dough stage 24 DAA (Zadoks stage 85). Spikes were harvested and stored on dry ice until storage at-80°C. Following storage, spikes were lyophilized and the seed threshed and weighed. Seeds were ground using a small plant grinder to pass a 40 mesh screen. At physiological maturity the entire plot was harvested. Homogeneity for a given *lpa* genotype was assessed by using a simple seed-crush assay as described by Larson et al. (2000).

Phosphorus Assays

Total P was assayed as described by (Larson et al., 2000; Raboy et al., 2000; and Dorsch et al., 2003). Briefly, between 200 and 250 mg of ground plant tissue were wet-ashed in 2 mL of concentrated sulfuric acid (H_2SO_4). Phosphorus concentration was determined colorimetrically (Chen et al., 1956). Inorganic P was extracted from 200–250 mg tissue using 12.5% TCA (v/w) in 0.5M $MgCl_2$ and measured colorimetrically using a plate reader (DynaTech Inc. Chantilly, VA). Phytic acid phosphorus was extracted using a method adapted from Haug and Lantzsch (1983). Samples (200 mg) were weighed into 15 mL screw cap conical tubes, and extracted overnight in 0.2N HCl. The samples were centrifuged and the 200 μL of the supernatant were transferred to a clean 15 mL conical bottom centrifuge tubes and diluted to 1 mL using 0.2N HCl. Ferric ammonium sulfate (1 mL) was added, and the tubes were boiled in a water bath for 15 min. The samples were cooled to room temperature and used for colorimetric determination of phytic acid phosphorus. Then 120 μL of the boiled sample were transferred to a well of a 96-well microtiter plate and 180 μL 1% 2,2′-bipyridine (w/v)-1% (v/v) thioglycolic acid were added for a total volume of 300 μl. The absorbance at 530 nm was read using a plate reader. Phytic acid standards were included to develop a standard curve between 1.5 to 24 μg/ml to calculate the amount of phytic acid in each sample. Because of the extremely low levels of $InsP_6$ in the M955 genotype, an ion exchange HPLC assay was used as described previously by Dorsch et al. (2003).

Non-phytate phosphorus content was estimated by subtraction of inorganic P and phytate P from the total P component. Data were expressed on a per seed basis as μg P seed^{-1} or on a concentration basis as mg P g^{-1}.

Table 1. Four barley *lpa* genotypes investigated in this study, the approximate reduction in phytic acid P and observations.

Mutant	Approximate Reduction in Phytic Acid P	Other Observations
lpa1–1	50%	Mutant phenotype is embryo specific. Increase in P_i only.
lpa2–1	50%	Accumulation of inositol phosphates with 5 and fewer phosphate esters, in addition to Pi accumulation. Similar to the maize lpa2–1.
lpa3–1	75%	Increase in P_i only.
M955	90%	Increase in P_i only.

Mineral Analysis

Mineral content was determined at the University of Idaho Analytical Sciences Laboratory, Moscow, ID. Content of As, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, K, S, V, and Zn were determined from ground tissue samples that were wet ashed in nitric acid and analyzed using a PerkinElmer Optima 3200 Inductively Coupled Plasma- Optical Emission Spectophotometer. The three sib-selections for each genotype were bulked in each of the two replicates. Analyses were performed from two field replicates of mature seed and seed harvested at 24 DAA.

Statistical Analysis

A mixed analysis of variance with fixed and random effects was conducted using PROC MIXED in SAS (Cary, NC). Replications within years were estimated as a random effects variable. Sampling time during harvest was treated as a repeated treatment using the REPEATED statement for PROC MIXED. Genotype values were estimated as the mean of two replicates with year for the three sibs of *lpa* or *wt* genotype pairs for each of the mutants studied. Least squares estimates of means (LSMEANS) were used for calculating genotype means and differences in comparisons between genotypes and sampling dates for seed weight, total phosphorus, inorganic phosphorus, and phytic acid phosphorus, and non-phytate phosphorus.

RESULTS AND DISCUSSION

The comparison of phosphorous accumulation within barley is described primarily on a per seed basis because several of the *lpa* genotypes differed from Harrington in this study. Seed weights for the four *lpa* genotypes and Harrington are shown in Table 2. The M955 genotype had a significantly lower seed weight than Harrington throughout development until physiological maturity.

Table 2. Dry seed weight of four *lpa* genotypes and Harrington over four harvest dates.

	10 Days		17 Days		24 Days		Maturity	
Genotype	mg seed^{-1}							
*lpa*1–1	10.5	ns	21.5	ns	38.5	ns	42.4	ns
*lpa*2–1	12.9	ns	22.3	ns	40.2	ns	39.9	**
*lpa*3–1	11.5	ns	19.6	ns	38.2	ns	40.7	*
M955	8.7	**	17.2	**	36.1	**	41.4	ns
Harrington	12.9		22.3		40.7		43.9	

*,** Significant at the 0.05 and 0.01 probability levels, respectively for comparisons of the *lpa* genotypes with Harrington.

The mutant lines *lpa*3–1 and *lpa*2–1 had significantly lower seed weight only at maturity (Table 2).

The content of total P, inorganic P, phytic acid P, and non-phytate P is summarized on per seed basis over the 2 yr in the study for four *lpa* genotypes and Harrington and is used for discussion of results (Fig. 1). Total phosphorus ranged between 27 μg P seed^{-1} to 163 μg P seed^{-1}. The general pattern of accumulation of total P was similar among all the genotypes in this study (Fig. 1). However, the *lpa*1–1 genotype had lower total P in mature seeds in 2003 than Harrington. This is similar to previous observations in mature seed (Dorsch et al., 2003). Total P content was similar in the 2 yr.

Inorganic P in mature seeds ranged from 12 μg P seed^{-1} in Harrington in 2004 to 98 μg P seed^{-1} in M955 in 2003. All genotypes were significantly different from each other in the mature seed and seed 24 DAA in both years, with the exception of *lpa*2–1 and *lpa*1–1, which were only significantly different in 2004. Inorganic P concentration increased during development of all lines except Harrington, which remained level during development. Inorganic P appears to decrease through 24 DAA when observed on a concentration basis (Data not shown). The rankings remain unchanged, but the *lpa* genotypes increase inorganic P after 24 DAA where Harrington continues to decrease.

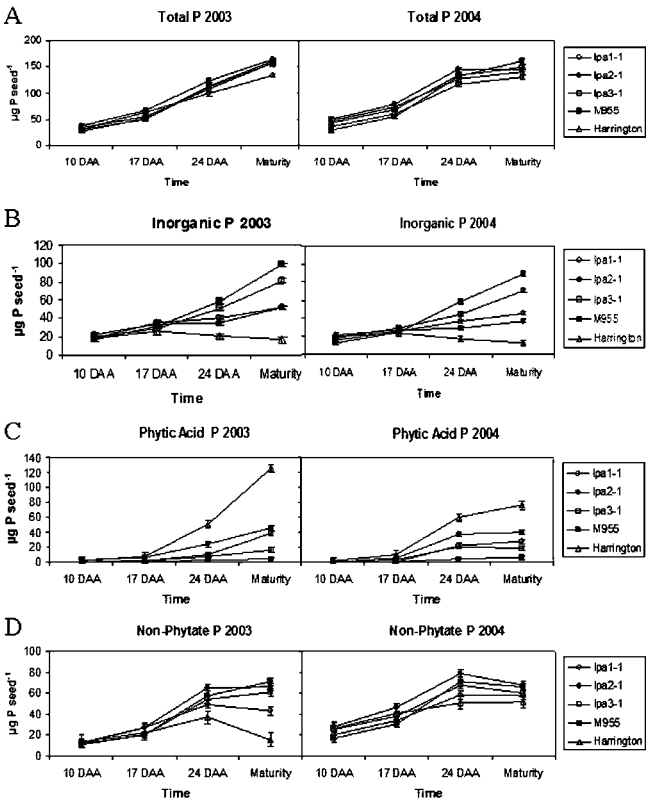

Fig. 1. Graph of seed content of (A) total P, (B) inorganic P, (C) phytic acid P, and (D) non-phytate P for four barley *lpa* genotypes, and Harrington from 2 yr. Expressed as μg P seed^{-1} over three dates during development and at maturity. P determinations as described in Materials and Methods. Data points and standard error bars represent means for two reps from three sib-selections of each *lpa* genotype, and two reps for Harrington.

Phytic acid P ranged from undetectable levels at 10 DAA to nearly 125 μg P seed^{-1} in Harrington in 2003. Yoshida et al. (1999) reported accumulation of phytin-containing globoids as early as 4 DAA in rice using microscopy; however we observed little measurable accumulation 10 DAA. M955 showed no significant differences throughout development, indicating that very little phytate accumulates in seeds of this genotype. The other genotypes showed only significant accumulation of phytate after 17 DAA. Phytate appears to accumulate in observable quantities later in development in the *lpa* lines than in Harrington. It is possible that accumulation is occurring in M955, albeit slowly and at levels below detection. The phytate P quantities within each *lpa* genotype were similar in 2003 and 2004. However phytate levels in Harrington were significantly lower in 2004 than in 2003.

The non-phytate phosphorus component ranged from 10 μg P seed^{-1} to 70 μg P seed^{-1}. M955, *lpa*2–1 and *lpa*3–1 showed significant decrease in non-phytate P in 2004 in mature seeds compared to Harrington. Non-phytate P also increased in all *lpa* genotypes at 24 DAA. The composition of this non-phytate phosphorus component is unknown and is an interesting avenue for further research.

Table 3 shows the analysis of variance and contrasts for the four phosphorus fractions for both years and in the individual years. The effect of years was significant ($P < 0.05$) for total P, inorganic P, and non-phytate P. The effect of week had significant quadratic trends for all analysis except for the combined years total P data and 2004 inorganic P. The two-way interactions for year*genotype and the three way interaction for year*week*genotype were highly significant for phytate P and non-phytate P, but nonsignificant for total P and inorganic P. Year*week interactions were significant for all traits.

Total P accumulation was similar in 2003 and 2004; however changes were observed in the distribution of the P components. The differences between years are evident with decreased inorganic P in 2004 *lpa* genotypes, reduced phytic acid in Harrington in 2004, and increased non-phytate P in Harrington and *lpa*1–1 in 2004. The *lpa* genotypes showed little change in phytate P and an increase in non-phytate P in 2004 relative to 2003. The differences between years in this study may be related to differing levels of available soil P, which was lower in 2004 than in 2003. Soil P measured 26 μg g^{-1} $NaCO_3$ extractable P in 2003 and 13 μg g^{-1} in 2004. This is similar to observations in dry beans (*Phaseolus vulgaris* L.) which had higher seed phytate levels with increased P fertility (Coelho et al., 2002). The major changes in phytate and non-phytate P occurred from 24 d to maturity, when phytate increased at a higher rate in soils with more available P (2003). Non-phytate P also appears to interact in the regulation of P homeostasis. Changes in seed P partitioning with differences in soil P levels also have been observed in soybeans (Oltmans et al., 2005) and sweet corn (*sugary1*; Tadmor et al., 2001). Despite lack of proper controls to test adequately this observation in barley, this work suggests that altered soil phosphate growing conditions also may influence accumulation of phosphorus fractions.

Table 3. Analysis of variance and contrasts in 2 yr for seed phosphorus fractions on a seed weight basis.

		Total P					Inorganic P					Phytic Acid P					Non-Phytate P				
		combined		2003		2004		combined		2003		2004		combined		2003		2004		combined		2003		2004	
Effect	*df*	*F*-Test																					
YEAR	1	51.9	*	*	*	*	*	81.4	*	*	*	*	*	5.0	ns	*	*	*	*	106.3	**	*	*	*	*
WEEK	3	2252.9	***	1097.8	***	1199.4	***	268.0	***	157.8	***	111.7	***	1699.4	***	1000.5	***	788.4	***	327.6	***	121.0	***	240.9	***
Linear	1	6593.3	***	3239.9	***	3392.8	***	783.3	***	456.1	***	330.0	***	4725.3	***	2584.4	***	2161.3	***	736.8	***	223.3	***	615.2	***
Quadratic	1	0.7	ns	27.7	***	21.3	***	4.3	*	4.2	*	0.8	ns	311.5	***	415.3	***	9.7	**	123.7	***	67.9	***	56.6	***
GENOTYPE	8	10.9	***	4.5	***	7.4	***	281.8	***	146.7	***	135.9	***	233.8	***	129.6	***	106.1	***	21.8	***	16.6	***	7.3	***
Harrington V *lpa*	1	0.1	ns	0.0	ns	0.2	ns	281.7	***	160.9	***	121.5	***	315.4	***	185.8	***	130.3	***	22.2	***	20.2	***	3.7	ns
Harrington V *wt*	1	1.3	ns	0.6	ns	0.7	ns	0.1	ns	0.3	ns	0.1	ns	5.3	*	4.1	*	1.4	ns	0.1	ns	0.2	ns	0.0	ns
lpa1–1 V Harrington	1	10.0	**	2.5	ns	8.7	**	120.9	***	73.6	***	48.1	***	228.1	***	130.7	***	98.0	***	4.5	*	7.3	**	0.0	ns
lpa2–1 V Harrington	1	8.1	**	5.0	*	3.1	ns	95.1	***	66.2	***	31.5	***	124.2	***	81.6	***	43.4	***	43.6	***	27.6	***	16.0	***
Lpa3–1 V Harrington	1	0.0	ns	0.0	ns	0.1	ns	313.5	***	165.4	***	148.1	***	299.3	***	184.7	***	115.4	***	15.7	***	13.8	***	2.9	ns
M955 V Harrington	1	0.4	ns	0.0	ns	0.6	ns	486.8	***	261.3	***	225.6	***	419.6	***	227.6	***	194.0	***	18.3	***	20.5	***	1.5	ns
YEAR*WEEK	3	29.8	***					3.4	*					124.8	***					7.5	***				
YEAR*GENOTYPE	8	0.7	ns					1.3	ns					5.7	***					4.1	***				
WEEK*GENOTYPE	24	3.3	***	2.0	**	2.4	***	111.7	***	59.0	***	53.8	***	78.7	***	53.1	***	29.7	***	8.1	***	8.4	***	2.4	***
lpa1–1 V Harr Linear	1	2.0	ns	4.3	*	0.1	ns	102.7	***	43.8	***	59.9	***	179.5	***	119.9	***	60.9	***	10.2	**	7.2	**	3.1	ns
lpa2–1 V Harr Linear	1	0.3	ns	0.1	ns	0.3	ns	54.5	***	32.8	***	22.0	***	128.1	***	100.5	***	32.1	***	28.5	***	25.3	***	5.1	*
Lpa3–1 V Harr Linear	1	1.3	ns	0.2	ns	1.6	ns	306.7	***	159.6	***	147.2	***	266.8	***	186.4	***	83.8	***	20.3	***	20.3	***	2.5	ns
M955 V Harr Linear	1	10.9	**	0.8	ns	15.7	***	587.8	***	273.6	***	316.1	***	366.7	***	232.4	***	135.7	***	42.6	***	31.0	***	12.3	***
YEAR*WEEK*GENOTYPE	24	1.0	ns					1.3	ns					8.3	***					4.0	***				

*, **, ***, Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Year to year differences in P distribution among the P fractions may have been caused by soil available P. This possibility was suggested in previous work in soybean (Raboy and Dickinson, 1993). The *lpa* genotypes and Harrington had different patterns in P partitioning in the 2 yr. It is possible that reduced capacity for phytate accumulation also reduces the ability to synthesize non-phytate phosphorus containing molecules. In P deficient soils the non-phytate P fraction increases, possibly due to sacrificing P flow into phytate and diverting it to non-phytate P-containing molecules. This highlights the need to investigate the actual composition of the non-phytate P fractions, the impact of varying soil available P on seed phosphorus composition, and the biological significance of the various P-containing metabolites.

Results of the mineral content analysis of barley seeds harvested at maturity and 24 d after anthesis are shown in Table 4. Results for As, Cd, Co, Cr, Pb, Mo, Ni, and V were at or below detection limits and were not presented. There were no significant year effects for any of the minerals in this study. Significant differences between seed at 24 DAA and maturity were observed for all minerals with the exception of Ca. Mineral content was higher in mature seed except for Na, which was lower in mature seed than seed at 24 DAA. Comparisons of mature seed of the *lpa* genotypes and Harrington showed significant differences in *lpa*1–1 and M955 for Cu, *lpa*1–1 for Fe and K, *lpa3–1* for Na, and M955 for Zn. In these cases Harrington had lower Cu and Fe content than the *lpa* genotypes, but higher K and Na content. At 24 d PAA, M955 and *lpa3–1* had significantly lower Na than Harrington. This differs from observations in wheat, where Cu and Zn were reduced in an *lpa* genotype compared to the *wt* (Guttieri et al., 2004). There were no other significant differences between the *lpa* genotypes and Harrington at this time point.

The *lpa* genotypes in this study represent a broad range of *lpa* phenotypes. The barley *lpa*1–1 genotype is aleurone specific, meaning the *lpa* phenotype is only evident in the aleurone seed fraction. The embryo displays the *wt* $InsP_6$ (Ockenden et al, 2004). The inositol phosphate seed phenotype of barley *lpa*2–1 is similar to the maize *lpa*2–1 in that it has a reduction in $InsP_6$, and accumulation of inositol phosphates with 5 and fewer phosphate esters (Dorsch et al., 2003). Mapping studies have mapped the barley *lpa*1–1 and *lpa*2–1 to barley chromosomes 2H and 7H, respectively (Larson et al., 1998). The mutant *lpa*3–1 has been mapped to 1HL (Roslinsky, 2002), as was M955. However they are not thought to be allelic (Roslinsky, unpublished data). The M955 genotype is of particular interest because of its extreme (> 90%) reduction in $InsP_6$. Maize mutants with an extreme phenotype such as this are lethal, and had to be maintained in the heterozygous state (Raboy, personal communications). Despite the large reduction in $InsP_6$, M955 is self-fertile and growth is not severely affected.

Modifications in phytate and phosphate accumulation in the low phytic acid mutants likely affect many other physiological processes. Multiple pathways such as cell wall synthesis and oligosaccharide production, and processes including cell signaling and stress response, utilize

Table 4. Mineral concentration in seeds of four barley *lpa* genotypes and *wt* line Harrington harvested at physiological maturity and 24 d after anthesis.

		Ca		Cu		Fe		Mg		Mn		K		Na		S		Zn	
		μg seed^{-1}																	
Year																			
	2003	**34.5**	**ns†**	**0.50**	**ns**	**1.4**	**ns**	**55.2**	**ns**	**0.87**	**ns**	**218.5**	**ns**	**10.2**	**ns**	**63.6**	**ns**	**1.6**	**ns**
	2004	**32.1**		**0.50**		**1.6**		**58.4**		**0.92**		**196.6**		**10.0**		**67.9**		**1.5**	
24 DAA		**33.9**	**ns‡**	**0.22**	*******	**1.1**	*******	**52.9**	*******	**0.86**	*****	**223.8**	*******	**11.8**	*******	**60.0**	*******	**1.4**	*******
	Harrington	**35.5**		**0.23**		**1.3**		**53.8**		**0.91**		**231.2**		**13.9**		**59.1**		**1.4**	
	***lpa*2–1**	**31.4**	**ns§**	**0.22**	**ns**	**1.2**	**ns**	**54.4**	**ns**	**0.86**	**ns**	**223.4**	**ns**	**14.2**	**ns**	**64.4**	**ns**	**1.4**	**ns**
	***lpa*1–1**	**31.9**	**ns**	**0.21**	**ns**	**1.0**	**ns**	**49.1**	**ns**	**0.77**	**ns**	**204.4**	**ns**	**12.4**	**ns**	**57.9**	**ns**	**1.3**	**ns**
	***lpa*3–1**	**38.2**	**ns**	**0.24**	**ns**	**1.1**	**ns**	**57.7**	**ns**	**0.94**	**ns**	**244.2**	**ns**	**11.0**	*****	**63.5**	**ns**	**1.4**	**ns**
	M955	**32.5**	**ns**	**0.21**	**ns**	**1.0**	**ns**	**49.6**	**ns**	**0.81**	**ns**	**215.8**	**ns**	**7.7**	*******	**55.1**	**ns**	**1.4**	**ns**
Maturity		**32.7**		**0.78**		**1.9**		**60.8**		**0.93**		**191.3**		**8.4**		**71.5**		**1.7**	
	Harrington	**33.7**		**0.73**		**1.7**		**61.0**		**0.95**		**206.4**		**9.9**		**72.1**		**1.7**	
	***lpa*2–1**	**31.5**	**ns**	**0.72**	**ns**	**1.9**	**ns**	**59.9**	**ns**	**0.93**	**ns**	**198.0**	**ns**	**7.9**	**ns**	**73.7**	**ns**	**1.8**	**ns**
	***lpa*1–1**	**32.5**	**ns**	**0.81**	*****	**2.2**	*****	**56.8**	**ns**	**0.87**	**ns**	**172.9**	*****	**9.8**	**ns**	**68.4**	**ns**	**1.6**	**ns**
	***lpa*3–1**	**32.5**	**ns**	**0.80**	**ns**	**1.8**	**ns**	**59.9**	**ns**	**0.93**	**ns**	**190.5**	**ns**	**6.9**	*****	**68.0**	**ns**	**1.7**	**ns**
	M955	**33.3**	**ns**	**0.85**	******	**1.9**	**ns**	**66.0**	**ns**	**0.98**	**ns**	**188.7**	**ns**	**7.7**	**ns**	**75.5**	**ns**	**1.9**	*****
Detection limit ug g^{-1}		**2.0**		**0.40**		**4.0**		**0.2**		**0.20**		**40.0**		**80.0**		**60.0**		**0.8**	

,**, Significant at the 0.05, 0.01, and 0.001 probability levels respectively for comparisons of the lpa genotype and Harrington.**
ns, not significant at $p = 0.05$.
† Probability for comparisons of data in 2003 with 2004.
‡ Probability for comparisons of mature seeds and seeds harvested 24 d after anthesis.
§ Probability for comparisons of the lpa genotype and Harrington.

inositol intermediates (Loewus and Murthy, 2000). Yield decreases can be an issue with greater deployment of *lpa* genotypes (Ertl et al., 1998). Although we noted no differences in emergence in this study, emergence was reduced in an earlier study of a soybean *lpa* genotype (Meis et al., 2003).

This study examined phosphate and phytate concentration during seed development. A similar study has been done in maize. In describing two maize *lpa* genotypes *lpa*1–1 and *lpa*2–1, Raboy et al. (2000) showed accumulation of total P, inositol P, and inorganic P during development. Results were similar, with total P changing little during seed development for *wt* and *lpa* lines. Inositol P increased steadily during development in the *wt* line, and in the *lpa*2–1, although the increase was much lower in *lpa*2–1, similar to our study. Inorganic P declined in the *wt* line but remained relatively constant in the *lpa* genotypes. We observed similar decreases in inorganic P in Harrington, and inorganic P decreased slightly during seed development of the *lpa* genotypes, but then increased in all the lpa genotypes from 24 d to maturity, with M955 and *lpa*3–1 having the largest increases in P_i.

From these results we reached several broad conclusions. First, the mutant phenotypes of the *lpa* genotypes are expressed throughout the development of the seed, with relative rankings among genotypes of phytic acid levels clearly evident at the earliest stages of phytic acid accumulation in the Harrington background. Second, the reduction in the synthesis of phytic acid in *lpa* barley resulted in greater concentrations of inorganic P, as is widely reported in the literature; yet this research also demonstrated increases within the *lpa* barley relative to the *wt* barley in amount of P fractions that are neither inorganic P nor phytic acid. The composition and biological function of this P fraction are open questions for future investigations. Finally, the environments used for this work significantly altered the percentage of P sequestered in phytic acid, in both *lpa* and *wt* barley. This suggests that further investigations could provide methods for enhancing the effects of *lpa* or *wt* by altering the field management of the crop, with soil P levels being the most promising variable for future crop management work.

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